

We claim:

1. A reagent for mass spectrometric analysis of proteins which has the general formula:



5 where A is an affinity label that selectively binds to a capture reagent, L is a linker group which can be differentially labelled with stable isotopes and PRG is a protein reactive group that selectively reacts with certain protein functional groups.

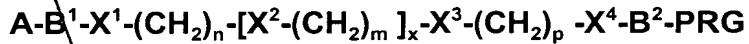
(2.) The reagent of claim 1 wherein PRG is a sulphhydryl reactive group or an amine reactive group.

(3.) The reagent of claim 1 wherein PRG is an enzyme substrate.

(4.) The reagent of claim 1 wherein the A-L-PRG is soluble in a sample liquid to be analyzed.

(5.) The reagent of claim 1 wherein the linker is a cleavable linker.

15 6. The reagent of claim 1 which has the general formula:



where: A is an affinity label;

PRG is a protein reactive group; and

20  $B^1-X^1-(CH_2)_n-[X^2-(CH_2)_m]_x-X^3-(CH_2)_p-X^4-B^2$  is a linker group wherein:

$X^1, X^2, X^3$  and  $X^4$ , independently of one another, and  $X^2$  independently of

other  $X^2$ , can be selected from O, S, NH, NR, NRR'+, CO, COO, COS, S-S, SO, SO<sub>2</sub>, CO-NR', CS-NR', Si-O, aryl or diaryl groups or  $X^1$ - $X^4$  may be absent;

5 B<sup>1</sup> and B<sup>2</sup>, independently of one another, are optional groups selected from COO, CO, CO-NR', CS-NR', (CH<sub>2</sub>)<sub>q</sub>-CONR', (CH<sub>2</sub>)<sub>q</sub>-CS-NR', or (CH<sub>2</sub>)<sub>q</sub>;

n, m, p, q and x are whole numbers that can take values from 0 to about 100, where the sum of n+xm+p+q is less than about 100;

R is an alkyl, alkenyl, alkynyl, alkoxy or an aryl group that is optionally substituted with one or more alkyl, alkenyl, alkynyl, or alkoxy groups; and

R' is a hydrogen, an alkyl, alkenyl, alkynyl, alkoxy or an aryl group that is optionally substituted with one or more alkyl, alkenyl, alkynyl, or alkoxy groups

wherein one or more of the CH<sub>2</sub> groups in the linker can be optionally substituted with alkyl, alkenyl, alkoxy groups, an aryl group that is optionally substituted with one or more alkyl, alkenyl, alkynyl, or alkoxy groups, an acidic group, a basic group or a group carrying a permanent positive or negative charge; wherein one or more single bonds linking non-adjacent CH<sub>2</sub> groups in the linker can be replaced with a double or a triple bond and wherein one or more of the atoms in the linker can be substituted with a stable isotope.

7. The reagent of claim 1 wherein the affinity label is biotin or a modified biotin.

8. The reagent of claim 1 wherein the affinity label is selected from the group consisting of a 1,2-diol, glutathione, maltose, a nitrilotriacetic acid group, or an oligohistidine.

9. The reagent of claim 1 wherein the affinity label is a hapten.

10. The reagent of claim 1 wherein PRG is a sulfhydryl-reactive group.

11. The reagent of claim 1 wherein PRG is an iodoacetylamide group, an epoxide, an  $\alpha$ -haloacyl group, a nitriles, a sulfonated alkyl, an aryl thiols or a maleimide.

5 12. The reagent of claim 1 wherein PRG is an amine reactive group, a group that reacts with a homoserine lactone or a group that reacts with carboxylic acid group.

13. The reagent of claim 1 wherein PRG is selected from the groups consisting of a amine reactive pentafluorophenyl ester group, an amine reactive N-hydroxy succinimide ester group, sulfonyl halide, isocyanate, isothiocyanante, active ester, tetafluorophenyl ester, an acid halide, and an acid anyhydride; a homoserine lactone reactive primary amine group, and an carboxylic acid reactive amine, alcohols or 2,3,5,6-tetrafluorophenyl trifluoroacetate.

14. The reagent of claim 1 wherein PRG is a substrate for an enzyme.

15. The reagent of claim 1 wherein PRG is a substrate for an enzyme the deficiency of which is associated with a birth defect.

16. The reagent of claim 1 wherein PRG is a substrate for an enzyme the deficiency of which is associated with a lysosomal storage disease.

20 17. The reagent of claim 1 wherein PRG is a substrate for  $\beta$ -galactosidase, acetyl- $\alpha$ -D-glucosaminidase, heparan sulfamidase, acetyl-CoA- $\alpha$ -D-glucosaminide N-acetyltransferase or N-acetylglucosamine-6-sulfatase.

18. The reagent of claim 1 wherein at least one of B1 or B2 is CO-NR' or CS-NR.

5

19. The reagent of claim 1 wherein  $X^1$  and  $X^4$  are selected from NH, NR, and  $NRR^+$ ,  $X^3$  is O and all  $X^2$  groups are O.

20. The reagent of claim 1 wherein the linker contains a disulfide group.

21. The reagent of claim 1 wherein any atom of the linker may be substituted with a heavy isotope.

22. A reagent kit for the analysis of proteins by mass spectral analysis that comprises a reagent of claim 1.

23. The reagent kit of claim 22 that comprises one or more reagents of claim 1.

24. The reagent kit of claim 22 further comprising one or more proteolytic enzymes for use in digestion of affinity tagged proteins.

25. The reagent kit of claim 22 which comprises a set of substantially chemically identical differentially labelled affinity tagged reagents.

26. The reagent kit of claim 22 wherein the reagent is an affinity tagged enzyme substrate reagent.

15 27. The reagent kit of claim 26 which comprises a set of substantially chemically identical differentially labeled affinity tagged enzyme substrates.

28. The reagent kit of claim 27 further comprising a set of substantially chemically identical differentially labeled affinity tagged enzyme products.